Phytoremediation of Heavy Metals (Ar, Cd, Pb) Using Transgenic Rice Plants - an Overview

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Abstract— Phytoremediation is the use of green plants to remove pollutants from the environment or render them harmless. Phytoremediation is a new technology in which plants are used to remove pollutants from water and soil. The use of metal-accumulating plants to clean environment contaminated with heavy metals is the most rapidly developing component of this environmentally friendly and cost-effective technology. The ever-increasing environmental pollution in agricultural soil caused by heavy metals due to application of sewage sludge, city refuse, and heavy metals containing fertilizers or pesticides, is becoming a major problem in modern agriculture. This review aims to provide a concise overview of plant metal tolerance and accumulation mechanisms. Another goal to present an overview of recent research considered to improve the phytoremediation capacity of plants by transgenic plants. Phytoremediation is based on the removal of contaminants from the soil by mechanisms such as phytoextraction, phytodegradation, rhizofiltration, phytostabilization and phytovolatilization through transgenic plants. This article reviews will provide more valuable information when phytoremediation process will be studied.

Index Terms— Environmental, Heavy Metals, Phytoremediation, Pollutants, Technology, Transgenic Plants

1 Introduction

Different types of heavy metals such as Cu, Zn and Ni are necessary micronutrients compulsory for a variety of functions including electron transfer reactions and as cofactors in many proteins and enzymes, on the other hand other metals like Ar, Cd and Pb are considered non-necessary. Both types of metals are toxic above certain concentrations. They inactivate metal-sensitive enzymes consequential in growth retardation and the worst in case of death of the organism. So, the metals found in our environment come from natural weathering process of earth's crust, soil erosion, mining, industrial discharge, urban runoff, sewage effluents, air pollution fall out, pest or disease control agents. The heavy metals also found by human activities such as application of phosphate fertilizers, military activities, metal working industries, mining and smelting.

The concentrations of the contaminants can vary from highly toxic concentrations from an inadvertent spill to barely measurable concentrations that after long-term exposure can be injurious to human health (Alexander, 1999). Mainly, heavy metals are toxic because they cause DNA damage and their carcinogenic effects in animals and humans are probably caused by their mutagenic ability (Knasmuller *et al.,* 1998). Heavy metals are not degradable without intervention stay in soil for centuries. As a result over recent decades an annual worldwide release of heavy metals reached 22,000 t (metric ton) for cadmium, 939,000 t for copper, 783,000 t for lead and 1,350,000 t for zinc.

Phytoremediation is based on the removal of contaminants from the soil by mechanisms such as phytoextraction, phytodegradation, rhizofiltration, phytostabilization and phytovolatilization (Salt *et al.,* 1995). The mechanisms of phytoremediation involved in heavy metal remediation are limited to uptake, adsorption, transport and translocation, sequestration into vacuoles, hyperaccumulation and in some cases, volatilization (Meagher, 2000). When present at increased concentrations, both essential (Cu, Fe, Mn, Mo, Zn) and non-essential metals (*e.g.*, Cd, Pd, Hg) are toxic. Heavy metals cannot be metabolized; only possible strategy to apply is their extraction from contaminated soil and transfer to the smaller volume of harvestable plants for their disposal (Padmavathiamma and Li, 2007).

Phytoremediation is an up-and-coming low-cost technology that utilizes plants to remove, transform, or stabilize contaminants including organic pollutants located in water, sediments, or soils. The advantages of phytoremediation over usual bioremediation by microorganisms are that plants, as autotrophic systems with large biomass, need only reserved nutrient input and they prevent the spreading of contaminants through water and wind erosion (Pulford and Watson, 2003). Plants also

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provide nutrients for rhizosphere bacteria, allowing the growth and maintenance of a microbial community for further contaminant detoxification.

A number of plant species have been recognized for the rationale of phytoremediation. Assured plant species known as hyper-accumulators are gorgeous candidates as they are able to accumulate potentially phytotoxic fundamentals to concentrations 50-500 times higher than average plants (Lasat, 2002). Tissue culture techniques are being increasingly practical for clonal multiplication and *in vitro* conservation of valuable indigenous which threatened with extinction (Roly *et al.,* 2013). Most workers that observed calli from mature seed were excellent starting material for transformation of rice by *Agrobacterium* due to their compactness (Islam *et al.,* 2013; Islam *et al.,* 2014; Islam *et al.,* 2014; Roly *et al.,* 2014). These transgenic rice plants are used for phytoremediation. The high bioconcentration factor and the efficient root to shoot transport system capable with enhanced metal tolerance supply hyper-accumulators with a high potential detoxification capacity (McGrath and Zhao, 2003).. However many of the hyper accumulators are slow growing and have reduced biomass production thus requiring several years for decontamination of the polluted sites. Trees appear as an attractive another due to their extensive root system, high water uptake, rapid growth, and large biomass production (Gullner *et al.,* 2001).

The remedial capacity of plants can be significantly improved using by genetic manipulation and plant transformation technologies (Van et al., 2004). The identification of unique genes from hyper-accumulators, and their subsequent transfer to fast-growing species have proven to be highly beneficial as demonstrated by the recent success of transgenic plants with enhanced phytoremediation capacity (Ellis *et al.,* 2004). This review goal to present an overview of recent research considered to improve the phytoremediation capacity of plants by transgenic plants. The use of transgenic plant species in phytoremediation programs is also discussed-

2 Phytoremediation by Transgenic Plant

Phytoremediation consists of mitigating pollutant concentrations in contaminated soils, water or air, with plants able to contain, degrade, or eliminate metals, pesticides, solvents, explosives, crude oil and its derivatives, and various other contaminants from the media that contain them. Molecular biology and genetic engineering are being increasingly considered as effective tools for better understanding and improving the phytoremediation capability of plants, whose biological functions can now be analyzed in detail and partly modified. The metal resistance systems are better known in microorganisms (Silver and Phung, 2005) in plants only a few systems of metal tolerance and/or sequestration are sufficiently characterized (Kärenlampi *et al.,* 2000).

In recent years, several key steps have been identified at the molecular level, allowing an increasing application of molecular-genetic technologies and a transgenic approach to a better understanding of mechanisms involved in heavy metal tolerance and accumulation in plants (Clemens *et al.,* 2002). It has been demonstrated in classic genetic studies that only a few genes are responsible for metal tolerance (Macnair *et al.,* 2000). Transfer and/or overexpression of genes responsible for metal uptake, translocation and sequestration may allow for the production of plants which, depending on the strategy, can be successfully exploited in phytoremediation (Krämer and Chardonnens, 2001).

Phytoremediation techniques were inadequate before twenty-five years ago. At the present time a lot of methods were discovered on phytoremediation with scientifically. The use of plants to decontaminate soils and waters has been developed only in recent times the first reports appearing in the eighties subsequently more comprehensive articles during the nineties (Cunningham *et al.,* 1995).

Phytoremediation technology has been recently extensively reviewed by some researchers (Dietz and Schnoor, 2001) and several species have been classified as hyperaccumulators and extensively investigated (Reeves, 1992). However, on a large scale, metal uptake by trees can be more effective, mainly because of a deeper root system and a greater yield of biomass (Greger and Landberg, 1999). High productivity and elevated uptake and translocation of pollutants to the harvestable biomass are the basis for efficient *in situ* restoration by means of vascular plants (Lasat, 2000). Some woody species can be advantageously used also for phytoremediation of soils along with groundwater from organic pollutants and hydrocarbons (Thompson *et al.,* 1998; Shaekh *et al.,* 2013). The potential in phytoremediation of metal contaminated soils expressed by forest trees has been assessed for several species in recent years (Arduini *et al.,* 1996). Resistance to metals often appears to be clone- or hybrid-specific rather than species-specific (Punshon and Dickinson, 1999).

Poplars are particularly suitable for remediation purposes, having already been considered for trials on metal tolerance in *in vivo* (Lingua *et al.,* 2005) and *in vitro* observations. Salicaceae are also reported to grow even in severe soil conditions and to accumulate heavy metals. Many studies have thus been focused on the use of willows and poplars in phytoextraction (Labreque *et al.,* 1995). These species can be advantageously exploited in short rotation coppice cultures (SRC), a strategy whose application in phytoremediation presents interesting and economically promising perspectives (Witters *et al.,* 2009). The inherent difficulties of experimenting on very large long-lived organisms such as forest trees, motivates the development of model systems. Besides the exploitation of hydroponic cultures, the *in vitro* model systems using shoot and cell cultures of plants demonstrated to be a useful tool for investigating efficiency of metal uptake and translocation. Cell and organ culture, in fact, as well as hydroponics, allow very fast accumulation of data in comparison with whole plant experiments under field conditions (Golan-Goldhirsh *et al.,* 2004), and offer the advantage of testing the effects of contaminants under controlled conditions (Harms, 1992). Hydroponic screening is often used to evaluate tolerance, accumulation and translocation in plants. A researcher demonstrated in *Salix* that results obtained in hydroponics and in field experiments are comparable (Watson *et al.,* 2003).

It is always advisable, however, to confirm data obtained by hydroponic tests by field performance trials. Using this technique, several studies have concerned, for instance, the response of willows to a metal cocktail and of willows and poplars to the presence of cadmium (Šottníková *et al.,* 2003), the response of a clone of *Populus* x *euramericana* to high concentrations of copper (Borghi *et al.,* 2007), the mechanism of resistance to aluminium of *Picea abies* (Heim *et al.*, 1999), the determination of the role of glutathione reductase metabolism in the defence of poplar (*Populus deltoides* x *P. nigra*) against high zinc concentration (Di Baccio *et al.*, 2005).

As stated by some of workers, the use of *in vitro* systems enables dissection of the complex system of plant, soil, and microbial interaction in order to evaluate the effect of stress factors on metabolism, specific enzymes and metabolites involved in plant response to the pollutant (Golan-Goldhirsh *et al.,* 2004). For many woody species, moreover, the application of *in vitro* propagation techniques, allows for fast plant production and the application of promising genetic engineering programs (Confalonieri *et al.*, 2003).

High concentration of zinc has been found to negatively affect the photosynthetic machinery in poplar: inhibition of adventitious root formation and leaf chlorosis indicated that the clone used was tolerant to external concentrations less than or equal to 1 mM, while in *Eucalyptus globulus* moderate concentrations of this metal were shown to either enhance or have no effect on rooting (Schwambach *et al.*, 2005). Phytoremediation potentials of poplar lines (*Populus nigra* and transgenic *P. canescens)* were investigated using *in vitro* leaf discs cultures and found that Zinc^{2+} as phytotoxic only at high concentrations (10^{-2} to 10^{-1} M) in all *P. canescens* lines, but *P. nigra* was more sensitive (Bittsanszky *et al.*, 2005). Cadmium added to the culture medium was shown to reduce the fresh and dry weights and the shoot length of white birch, while root length was not affected (Fernández *et al.*, 2008). Copper at a concentration of 0.05 mM, manganese at 0.80 mM, and zinc at 0.12 mM showed a negative effect on shoot growth (number of shoots per explant and shoot length) in *Ailanthus altissima*, considered a fast-growing and contamination-resistant species (Gatti, 2008). Zinc was found toxic in aspen (*Populus tremula* x *tremuloides*) cultures at 0.5 mM concentration, while lead at the same concentration did not show toxic effects and was accumulated at 3500 µg per g of biomass (Kalisova-Spirochova *et al.,* 2003).

In vitro studies were also developed to investigate the effects of high concentrations of zinc and copper on the biosynthesis and accumulation of polyamine in *Populus alba* (Franchin *et al.*, 2007). On the basis of leaf symptoms, rate of adventitious root formation and ethylene production, it was found that Zn at 0.5-1 mM concentration was transiently toxic, while at 2-4 mM was increasingly toxic. Free and conjugated putrescine and spermidine accumulated proportionally to toxicity; also Cu strongly reduced rooting already at 5 µM and caused severe, dose-dependent toxicity symptoms (shoot chlorosis and necrosis) using concentrations up to 500 µM. In *in vitro* growing microshoots of *Populus alba*, the effect of high concentrations of cadmium, copper, zinc, and arsenic was investigated, showing differences in the response of different clones (Di Lonardo *et al.,* 2011). Axenic poplar tumor cell cultures were tested for demonstrating the capability of taking up trichloroethylene (TCE) and degrading it to several known metabolic products (Newman *et al.,* 1997), while poplar (*Populus deltoides×P. nigra*) *in vitro* culture has been used for developing mathematical models to define degradation pathways of nitramine compounds within plant cells (Mezzari *et al.,* 2004). Metal tolerance was detected in a callus culture established from *Acer rubrum* seedlings growing in soil contaminated by zinc, cadmium, nickel and arsenic. A positive linear correlation was found between zinc resistance of callus and total Zn in soil beneath sampled trees, while no significant correlations were evidenced with the other metals (Watmough and Hutchinson, 1998). In *Acer pseudoplatanus* callus culture, Cu-, Zn- and Cd-resistance traits were identified in cell lines originating from trees at a site contaminated by these metals (Watmough and Dickinson, 1995).

In vitro screenings were also used to investigate how several heavy metals affect pollen germination and tube elongation in *Pinus resinosa* (Chaney, 1983) and to test the tolerance for Zn and Cu in mycorrhyzal isolates collected in an abandoned Cu mines, in view of their inoculation into *Pinus sylvestris* seedlings (Adriaensen *et al.,* 2005). Combined micropropagation and hydroponic culture were used to study tolerance to copper and zinc in *Betula pendula*, finding that a seed-derived clone from a Pb/Zn-contaminated site showed more tolerance to Cu and Zn than bud-derived clones from a Cu/Ni-contaminated site or from an uncontaminated area (Utriainen *et al.,* 1997).

3 Conclusions

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Phytoremediation of metal-polluted soil by plant phytoextraction is a technique attracting the interest of an increasing scientific community and the use of transgenic plants, in particular, presents some aspects of relevance. Biotechnologies are surely powerful tools allowing investigating and evaluating the potential of phytoremediation. As described in this paper, many fields of study are contributing to a rapid increase of our knowledge on the mechanisms involved. However, despite of the intensive research carried out in the last years on this topic, very few field trials demonstrated the technical feasibility and economic workability of the described approaches. Indeed, most of the literature rarely provides information on the practical application of phytoremediation techniques. Specialization and fragmentation of research is probably real, but it should not be seen as a limit: every progress can contribute and converge to increase the possibility of an advantageous exploitation of transgenic plants for phytoremediation.

References

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A. Bittsanszky, T. Komives, G. Gullner, G. Gyulai, J. Kiss, L. Heszky, L. Radimszky, H. Rennenberg, "Ability of transgenic poplars with elevated glutathione content to tolerate zinc(2+) stress," *Environment International*, vol. 31, pp. 251-254, 2005.

A. Dietz, J.L. Schnoor, "Advances in phytoremediation," *Environmental Health Perspectives*, vol. 109, pp. 163-168, 2001.

A. Golan-Goldhirsh, O. Barazani, A. Nepovim, P. Soudek, S. Smrcek, and L. Dufkova, "Plant response to heavy metals and organic pollutants in cell culture and at whole plant level," *Journal of Soils and Sediments*, vol. 4(2), pp. 133-140, 2004.

A. Heim, J. Luster, I. Brunner, B. Frey, and E. Frossard E, "Effects of aluminium treatment on Norway spruce roots, aluminium binding forms, element distribution, and release of organic acids," *Plant and Soil*, vol. 216, pp. 103-116, 1999.

A. Šottníková, L. Lunácková, E. Masarovicová, A. Lux, V. Streško, "Changes in the rooting and growth of willow and poplars induced by cadmium," *Biologia Plantarum*, 46: 129-131, 2003.

A.A. Watmough, T.C. Hutchinson, "Relationship between soil metal contamination and metal resistance of callus established from mature red maple," *Canadian Journal of Forest Research*, 28(7): 1073-1077, 1998.

C. Franchin, T. Fossati, E. Pasquini, G. Lingua, S. Castiglione, and P. Torrigiani, "High concentrations of Zn and Cu induce differential polyamine responses in micropropagated white poplar (*Populus alba* L. 'Villafranca')," *Physiologia Plantarum*, vol. 130, pp. 77-90, 2007.

C. Watson, I.D. Pulford, D. Riddell-Black, "Screening of willow species for resistance to heavy metals: Comparison of performance in a hydroponics system and field trials," *International Journal of Phytoremediation*, 5: 351-365, 2003.

Clemens S, Palmgren MG, Kranmer U. 2002. A long way ahead: understanding and engineering plant metal accumulation. Trends in Plant Science. 7: 309-314.

D. Di Baccio, S. Kopriva, L. Sebastiani, H. Rennenberg, "Does glutathione metabolism have a role in the defence of poplar against zinc excess?," *New Phytologist*, vol. 167, pp. 73-80, 2005.

D.E. Salt, R.D. Smith, I. Raskin I, "Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants," *Biotechnology*, 13: 468-474, 1995.

D.R. Ellis, T.G. Sors, D.G. Brunk, C. Albrecht, C. Orser, B. Lahner, K.V. Wood, H.H. Harris, I.J Pickering , and D.E. Salt, "Production of Se-methylselenocysteine in transgenic plants expressing selenocysteine methyltransferase," *BMC Plant Biol*, vol. 4, pp. 318–333, 2004.

E. Gatti, "Micropropagation of *Ailanthus altissima* and *in vitro* heavy metal tolerance," *Biologia Plantarum*, vol. 52(**1**), pp. 146-148, 2008.

G. Gullner, T. Komives, H. Rennenberg, "Enhanced tolerance of transgenic poplar plants overexpressing g-glutamylcysteine synthetase towards chloroacetanilide herbicides," *J. Exp. Bot*, vol. 52, pp. 971-979, 2001.

G. Lingua, S. Castiglione, V. Todeschini, C. Franchin, T. Fossati, E.A. Peterson, "Selection of elite poplar clones for phytoremediation of soil contaminated by heavy metals in field and glass-house experiments," In: Proceedings of the "XVII International Botanical Congress", Wien (Austria). pp. 624, 2005.

H.H. Harms, "*In vitro* systems for studying phototoxicity and metabolic fate of pesticides and xenobiotics in plants," *Pesticide Science*, vol. 35, pp. 277-281, 1992.

I. Arduini, D.L. Godbold, A. Onnis, "Cadmium and copper uptake and distribution in Mediterranean tree seedlings," *Physiologia Plantarum*, vol. 97, pp. 111-117, 1996.

I. Kalisova-Spirochova, J. Puncocharova, Z. Kafka. 2003. Accumulation of heavy metals by *in vitro* cultures of plants. *Water, Air and Soil Pollution*. 3: 269-276.

I.D. Pulford, C. Watson, "Phytoremediation of heavy metal-contaminated land by trees - A review," *Environment International*, 29: 529-540, 2003.

J. Schwambach, C. Fadanelli, A.G. Fett-Neto, "Mineral nutrition and adventitious rooting in microcuttings of *Eucalyptus globules, Tree Physiology*," 25: 487-494, 2005.

K. Adriaensen , T. Vrålstad, J.P. Noben, J. Vangronsveld, and J.V. Colpaert, "Copper adapted *Suillus luteus*, a symbiotic solution for pines colonising Cu mine spoil," *Applied and Environmental Microbiology*, vol. 71, pp. 7279-7284, 2005.

L.A. Newman, S.E. Strand, N. Choe, J. Duffy, G. Ekuan, M. Ruszaj, "Uptake and biotransformation of trichloroethylene by hybrid poplars," *Environmental Science and Technology*, 31: 1062-1067, 1997.

M. Alexander, "Biodegradation and bioremediation", Ithaca, NY, USA: Academic Press, 1999.

M. Borghi, R. Tognetti, G. Monteforti, L. Sebastiani, "Responses of *Populus* x *euramericana* (*P. deltoides* x *P. nigra*) clone *Adda* to increasing copper concentrations," *Environmental and Experimental Botany*, vol. 61, pp 66-73, 2007.

M. Confalonieri, A. Balestrazzi, S. Bisoffi, D. Carbonera, "*In vitro* culture and genetic engineering of *Populus* spp.: synergy for forest tree improvement," *Plant Cell Tissue and Organ Culture,* vol. 72, pp. 109-138, 2003.

M. Greger, and T. Landberg , "Use of willow in phytoextraction," *International Journal of Phytoremediation*, vol. 1, pp. 115-123, 1999.

M. Labreque, T.I. Teodorescu, S. Daigle, "Effect of wastewater sludge on growth and heavy metal bioaccumulation of two *Salix* species," *Plant and Soil,* 171: 303-316, 1995.

M.A. Utriainen, L.V. Kärenlampi, S.O. Kärenlampi, H. Schat, "Differential tolerance to copper and zinc of micropropagated birches tested in hydroponics," *New Phytologist,* 137: 543-549, 1997.

M.M Islam, M.E. Haque, M.A. Islam MA, B. Sikdar, M. Khalekuzzaman. Establishment of an Efficient Protocol for *In Vitro* Callus Induction and Regeneration System Using Mature Embryo in Elite Rice (*Oryza sativa* L.) Cultivars. *Research in Plant Biology*, 4(4): 09-20, 2014.

M.M. Islam, M.E. Haque, S.M.M. Alam, M.A. Islam, M. Khalekuzzaman , and B. Sikdar, "Morphological and Histological Observation of Embryogenic Calli Derived from Immature Embryo of BRRI Dhan28 (*Oryza sativa* L.) Variety," *Res. Plant Biol,* 3(5), 21-27, 2013.

M.M. Islam, Z.Y. Roly, Y. Lee Y, M. Khalekuzzaman, "*In vitro* Propagation and Genetic Transformation System Using Immature Embryo in Elite Rice (*Oryza sativa* L.) Cultivars," *The Korean Society of Breeding Science,* 2(1): 88-96, 2014.

M.M. Lasat, "Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues," *J. Hazard. Subst. Res. 2: 1-25, 2000.*

M.M. Lasat, "Phytoextraction of toxic metals: a review of biological mechanisms," *Journal of Environmental Quality,* 31: 109-120, 2002.

M.P. Mezzari, B. Van Aken, J.M. Yoon, C.L. Just, J.L. Schnoor, "Mathematical modeling of RDX and HMX metabolism in poplar (*Populus deltoides* x *Populus nigra*, DN34) tissue culture," *International Journal of Phytoremediation*, 6: 323-345, 2004.

M.P.E. Shaekh, A. Mondol, M.M. Islam, A.S. Kabir, M.A. Saleh, M. Salah Uddin, K.M.F. Hoque, and A.E. Ekram, "Isolation, Characterization and Identification of an Antagonistic Bacterium from *Penaeus monodon," International Journal of Scientific & Engineering Research,* 4(10), pp. 254-261, 2013.

M.R. Macnair, G.H. Tilstone, S.E. Smith, "The genetics of metal tolerance and accumulation in higher plants, In: Phytoremediation of contaminated soil and water," (Terry N, Bañuelos G eds). Lewis Publications, Boca Raton, FL, USA, pp. 235-250, 2000.

N. Witters, S. Van Slycken, A. Ruttens, K. Adriaensen, E. Meers, L. Meiresonne, "Short-rotation coppice of willow for phytoremediation of a metal-contaminated agricultural area: a sustainability assessment," *BioEnergy Research*, 2(3): 144-152, 2009.

P.K. Padmavathiamma, L.Y. Li, "Phytoremediation technology: hyper- accumulation metals in plants," *Water, Air and Soil Pollution,* 184: 105-126, 2007.

P.L. Thompson, L.A. Ramer, J.L. Schnoor, "Uptake and transformation of TNT by hybrid poplar trees," Environmental Science and Technology, 32: 975-980, 1998.

R. Fernández, A. Bertrand, A. Casares, R. García, A. González, and R.S. Tamés, "Cadmium accumulation and its effect on the in vitro growth of woody fleabane and mycorrhized white birch," *Environmental Pollution*, vol. 152 (3), pp. 522-529, 2008.

R.B. Meagher, "Phytoremediation of toxic elemental and organic pollutants," *Current Opinion in Plant Biology*, 3: 153-162, 2000.

ISSN 2229-5518
R.D. Reeves, "The hyperaccumulation of nickel by serpentine plants. In: The vegetation of ultramafic (Serpentine) soils," (Baker AJM, Proctor J, Reeves RD eds), Intercept Ltd, Andover, UK, pp. 253-277, 1992.

R.L. Chaney, Plant uptake of inorganic waste constitutes In: Land treatment of hazardous wastes (Parr JF, Marsh PB, Kla JM eds). Noyes Data Corp., Park Ridge, USA, pp. 50-76, 1983.

S. Di Lonardo, M. Capuana, M. Arnetoli, R. Gabbrielli, C. Gonnelli, "Exploring the metal phytoremediation potential of three *Populus alba* L. clones using an in vitro screening," *Environmental Science and Pollution Research,"* vol. 18 pp. 82-90, 2011.

S. Kärenlampi, H. Schat, J. Vangronsveld, J.A.C. Verkleij, D. Van der Lelie, M. Mergeay. 2000. Genetic engineering in the improvement of plants for phytoremediation of metal polluted soils. *Environmental Pollution.* 107: 225-231.

S. Knasmuller, E. Gottmann, H. Steinkellner, A. Fomin, C. Pickl, A. Paschke, R. God, M. Kundi, "Detection of genotoxic effects of heavy metal contaminated soils with plant bioassays," *Mutation Research*, 420: 37-48, 1998.

S. Silver, L.T. Phung, "A bacterial view of the periodic table: genes and proteins for toxic inorganic ions," *Journal of Industrial Microbiology and Biotechnology*, 32: 587-605, 2005.

S.A. Watmough, N.M. Dickinson, "Multiple metal resistance and co-resistance in *Acer pseudoplatanus* L. (sycamore) callus cultures," *Annals of Botany*, 76: 465-472, 1995.

S.D. Cunningham, W.R. Berti, J.W. Huang, "Phytoremediation of contaminated soils," *Trends in Biotechnology*, vol. 13(9), pp. 393-397, 1995.

S.P. McGrath, F.J. Zhao, "Phytoextraction of metals and metalloids from contaminated soils," *Curr. Opin. Biotechnol,* 14: 277-282, 2003.

T. Punshon, N.M. Dickinson, "Heavy metal resistance and accumulation characteristics in willows," *Internationl Journal of Phytoremediation*, 1: 361-385, 1989.

T. Van, N. Terry, E.A. Pilon-Smits, "Exploring the selenium phytoremediation potential of transgenic Indian mustard overexpressing ATP sulfurylase or cystathioninegamma- synthase," Int. J. Phytorem. 6: 111-118, 2004.

U. Krämer, A.N. Chardonnens, "The use of transgenic plants in the bioremediation of soils contaminated with trace elements," *Applied Microbiology and Biotechnology*, 55: 661-72, 2001.

Z.Y. Roly, M.M. Islam, M.P.E. Shaekh, M.S.I. Arman, S.M. Shahik, D. Das, M.M.E. Haamem, M. Khalekuzzaman, "*In vitro* callus induction and regeneration potentiality of aromatic rice (*Oryza sativa* L.) cultivars in differential growth regulators," *Int J Appl Sci Biotechnol*, 2(2): 156-163, 2014.

Z.Y. Roly, M. Mahmudul, M.E. Haque, M.A. Islam, B. Sikdar, "*In vitro* multiple shoot regeneration from cotyledonary nodes, shoot tips and nodal segments of ridge gourd," *Discovery Biotechnology*, 4(9): 4-8, 2013.